were also created to test the role of B cells in a Th1-mediated colitis that develops in the absence of IL-10. **RESULTS:** At six months of age, αIL10DKO and αμIL10TKO mice developed more severe colitis compared to TCRαKO mice. There was no significant difference in the disease scores between αIL10DKO (5.47 ± 3.2) and αμIL10TKO (5.93 ± 1.9) mice as judged by gross and histological examinations. However, all αμIL10TKO mice developed colitis, while, like TCRα KO mice, 30% of αIL10DKO mice remained colitis free. These findings suggest that IL-10 produced by B cells contributes to the regulation of on-going colitis, whereas other B cell factors are involved in the suppression of colitis development. To extend these findings to Th1 mediated colitis, we generated IL-10 and B cell double knockout (IL10μDKO) mice. In contrast to findings in TCRα KO mice, the severity as well as frequency of colitis in IL-10 KO mice was identical to those in IL10μDKO mice. **CONCLUSION:** These results suggest that B cells regulate Th2, but not Th1-mediated colitis, by both IL-10-dependent and IL-10-independent pathways; IL-10-independent regulation is involved in the colitis development, whereas, IL-10-dependent regulation is involved in the progressive phase (exacerbation) of colitis.

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Microbial Antigens Stimulate Expression of Epstein-Barr Virus Induced-Gene (ebi-)3 and Other Il-12 Related Molecules in Human Intestinal Microvascular Endothelial Cells (HIMEC)

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AIMS: Members of the interleukin-12 (IL-12) family, which include IL-12p35, p19, IL-12p40, Epstein–Barr virus-induced gene (EBI)-3 and p28 constitute subunits of IL -12, -23, and -27. These ILs represent pivotal mediators in the regulation of cell-mediated immune responses. Recent work has suggested that intestinal endothelial cells could serve as a second line of defense in bacterial sensing of invading pathogens. The purpose of this study was to examine the production of IL-12 family members in HIMEC as part of their inflammatory responses after stimulation with microbial pathogens. **METHODS/MATERIALS:** HIMEC were isolated from normal human resected bowel specimens and assessed for culture purity, as described earlier (Binion et al., Gastroenterology 1997). HIMEC were stimulated with proinflammatory agents including endogenous mediators (TNF-α, IFN-γ, IL-1β) and microbial antigens (LPS, PGN, CpG-DNA, ds-RNA, LAM, flagellin), as endothelial cells are known to express the corresponding Toll-like receptors. Production of IL-12 family members in HIMEC was assessed by real time RT-PCR, indirect immunofluorescence staining, and immunoblot analysis of cell lysates and culture supernatants. PBMC stimulated with LPS served as a positive control. **RESULTS:** HIMEC display a strong inducibility of EBI-3 and IL-12p35, and to a lesser extent, of p19, as assessed by real time RT-PCR. Neither constitutive nor inducible expression of IL-12p40 and p28 could be detected, as compared to positive control. The most pronounced regulation of EBI-3 (100-fold by TNF-α) and IL-12p35 (80-fold by TNF-α) was induced by proinflammatory factors known to utilize the NFκB activation pathway in endothelial cells. Consequently, stimulated EBI-3 and IL-12p35 gene expression was diminished by MG132, a NFκB inhibitor, in a dose dependent manner. In addition to mRNA expression, EBI-3 protein expression in HIMEC was strongly up-regulated, as detected by immunoblot analysis. Furthermore, immunofluorescence analysis revealed that EBI-3 protein was redistributed to the HIMEC cell surface upon stimulation with TNF-α. **CONCLUSION:** Our data indicate that HIMEC are capable of producing IL-12 family members, mediators crucially involved in mucosal antigen presentation, as a response to microbial stimuli. The intestinal microvasculature might thus serve functions as a second line of defense in adaptive and innate immune functions, including bacterial sensing and antigen presentation.

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The Interleukin-8-251 Promoter Polymorphism and Risk of Gastric Cancer in Caucasian and Japanese Populations.

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Background: Interleukin 8 is of critical importance in the inflammatory response to *Helicobacter pylori*. It is a powerful chemotactic factor that reduces many of the early inflammatory responses to the infection. We have recently shown that a functional promoter polymorphism (IL-8–251 A/T) is associated with an increased risk of developing the pre-malignant changes of hypochlorhydria and gastric atrophy. We have also demonstrated that carriage of the IL-8–251 A allele is associated with higher IL-8 levels and a more pronounced inflammatory response in the gastric mucosa.

Aim: To evaluate the effect of the IL-8–251 (A/T) polymorphism on the risk of developing gastric carcinoma, using case control studies from two populations of differing ethnic backgrounds.

Subjects and Methods: We used a 5' nuclease assay to genotype the IL-8–251 A/T polymorphism in two gastric cancer case control studies: 1) a Caucasian gastric cancer case-control study consisting of 306 gastric cancer cases and 211 controls and, 2) a Japanese gastric cancer case-control study consisting of 237 gastric cancer cases and 98 controls. Odds ratios and 95% confidence intervals (CI) were calculated and logistic regression was used to adjust for confounding variables.

Results: Carriage of the pro-inflammatory IL-8–251 A allele in the Caucasian case-control study was not associated with an increased risk of developing gastric carcinoma (OR = 1.006, 95% CI 0.7 – 1.5). No significant differences were observed when the cases were subdivided into cardia (OR = 0.811, 95% CI 0.5 – 1.3) and non-cardia gastric cancers (OR 1.173, 95% CI 0.8 – 1.8). Similarly in the Japanese population carriage of the A allele did not increase the risk of having gastric cancer (OR = 1.166, 95% CI 0.7 – 1.9).

Conclusion: Although carriage of the IL-8–251 A allele is associated with a more pronounced inflammatory response in the gastric mucosa of *H. pylori* infected subjects and an increased risk of developing pre-malignant changes, it does not appear to alter the risk of developing the eventual outcome of gastric cancer. This applies to populations of differing ethnicity. We postulate that this polymorphism is important at an early stage in the inflammatory response to *H. pylori* and may facilitate the action of other mediators in the development of gastric cancer.

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Inhibition of the Tff1 Gene and Activation of Stat3 Are Early Events Which May Predispose To Neoplasia Following *Helicobacter Pylori* Infection of the Human Stomach

Cameron Jackson

Helicobacter pylori (HP) infection of the stomach accounts for most cases of human gastric cancer, however events underlying disease initiation are unknown. Our mouse model of stomach tumourigenesis demonstrates that an imbalance in IL-6 family cytokine signaling pathways produces homeostatic dysregulation. Events preceding tumour development include the reduced expression of the tumour suppressor gene *tff1*, as a result of a mutation in the SHP2/SOCS3 binding site on the β receptor gp130. Ablated binding SHP-2 and the negative reulator of STAT3, SOCS3 to gp130 results in attenuated MAP kinase signaling, and constitutive activation of oncogenic STAT3. Recently it has been shown that CagA, an HP cytotoxin is injected into human gastric epithelial cells where similarly disrupts MAP kinase signaling by constitutively binding SHP-2. Therefore CagA+ HP infection of human stomach may phenocopy our mouse model such that IL-6-driven regulatory pathways may be altered resulting in impaired TFF1 protein synthesis and constitutive activation of STAT3. In order to test this, we analysed gene expression by Northern (*tff1*) and Western (activated phopho-STAT3) analysis in human gastric biopsies from patients with and without *H. pylori* infection. The CagA status of the biopsies was confirmed by Western analysis. In HP-infected biopsies, phosphorylated (activated) STAT3 was increased 3.5 fold in antrum ($p = 0.046$), and > 20 fold ($p = 0.0001$) in mid-body mucosa, compared to uninfected controls. Moreover gastric biopsies infected with CagA+ HP, had much greater activation of STAT3 ($p = 0.033$) than CagA- biopsies. In addition, Northern analysis of *tff1* expression (*tff1*: L32) showed significantly decreased *mRNA* in HP-colonised compared to control biopsies in antrum (99%, $p = 0.057$) and midbody mucosa (80%, $p = 0.008$). Together our data suggest that following *H. pylori* infection, IL-6 regulated signaling pathways are imbalanced, causing suppression of *tff1* gene expression and enhanced STAT3, particularly in the presence of CagA. However no consistent changes in IL-6 or IL-11 expression were observed in the biopsies, suggesting that ablated SHP-2/SOCS3, and increased STAT3 signaling are independent of cytokine expression, and is most likely due to infection with CagA+ HP. These constitutive early events might contribute to compromised gastric mucosal homeostasis and promote tumourigenesis in susceptible individuals.

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Gastric Intraepithelial Neoplasia in *Helicobacter Pylori* Infected B6129 Mice Is Not Promoted By a High Salt Diet

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Helicobacter pylori underlies most cases of stomach cancer. Gastric carcinoma is highly prevalent in human populations consuming high salt diets. Attempts to test the hypothesis that high salt promotes *H. pylori* carcinogenesis have been hindered by the lack of a proven wild-type (WT) mouse model. Gastric cancer in *H. pylori*-infected transgenic INS-GAS mice is not potentiated by high salt. However, effects of salt in this system may be masked by supraphysiologic gastrinemia. We have observed marked hyperplasia and dysplasia in C57BL/6 x 129/SvEv (B6129) mice with chronic gastritis. The dual aims of this study were to characterize *H. pylori* infection in B6129 mice, and to determine whether high salt promotes tumorigenesis. We divided 174 B6129 mice into 4 groups. At 8 weeks, mice were gavaged with 10^9 colony-forming units (CFU) *H. pylori* Sydney strain 1 or vehicle only, and maintained on a basal (0.25%) or high (7.5%) salt diet until necropsy at 6, 12, or 15 months. Colonization was assessed by quantitative culture, and histologic lesions scored by a comparative pathologist blinded to treatment groups. In uninfected mice, high salt increased inflammation, oxyntic atrophy, and mucous metaplasia scores. Infected mice on the high salt diet maintained significantly higher *H. pylori* burdens than did mice on the basal diet (109,634 vs. 9474 CFU/g stomach respectively, $P < 0.001$). Histopathology of infected mice demonstrated progressive inflammation, oxyntic atrophy, hyperplasia, intestinal metaplasia and dysplasia, recapitulating Correa's progression. Mucous metaplasia and oxyntic atrophy were associated with high salt, but a statistically insignificant trend towards higher scores for other criteria was seen in mice on the basal diet. No significant gender differences were noted. At 15 months, infected mice in both dietary groups exhibited high-grade gastric intraepithelial neoplasia or carcinoma in situ. In summary, high salt is associated with significantly increased *H. pylori* colonization in B6129 mice. High salt induces mild atrophic gastritis, but no additive effect is observed with *H. pylori* gastritis. We plan to test the hypothesis that type 1 cytokines upregulated by high salt injury antagonize type 1 immune responses to *Helicobacter* infection. Importantly, we report for the first time *H. pylori*-induced high-grade gastric intraepithelial neoplasia in wild-type mice. The B6129 model provides a new opportunity to study *H. pylori* carcinogenesis in vivo.

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A COX-2 Promoter Polymorphism Increases the Risk of Developing Pre-Malignant Changes in a Population of Healthy First-Degree Gastric Carcinoma Relatives.

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Background: Cyclooxygenase-2 (COX-2) plays a number of key roles in carcinogenesis including stimulation of cellular proliferation and angiogenesis and inhibition of apoptosis. COX-2 expression is upregulated in gastric pre-malignant lesions and adenocarcinomas and increased expression has been correlated with poor clinicopathological variables. Single nucleotide polymorphisms have been described in the COX-2 gene: 3 promoter polymorphisms (-1195G>C, -765G>C and -899G>C), one exonic (Exon 3 8473T>C) and one within the 3-untranslated region (3' UTR 1>C). All polymorphisms are potentially functional, in